EXHIBIT A

CURRICULUM VITAE

I. PERSONAL

A. Name: John J. Donnelly

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Moraga, CA 94556

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II. EDUCATION

School	Date	Field	Degree
University of Pennsylvania	1971-1975	Biology	B.A.
University of Pennsylvania	1975-1979	Immunology	Ph.D.
US Army War College	2000-2002	Strategic studies	M.S.

III. TRAINING

Source	Date	Type
Department of Ophthalmology John Hopkins University School of Medicine Baltimore, Maryland	1982	Postdoctoral Research Fellow (Preceptor: R.A. Prendergast, M.D.)
Department of Clinical Veterinary Medicine University of Cambridge Cambridge, England	1980-81	Postdoctoral Research Fellow (Preceptor: Prof. E.J.L. Soulsby, D.V.S.M., M.R.C.V.S., Ph.D.)

IV. SOCIETY MEMBERSHIPS

American Association of Blood Banks
American Association of Immunologists
Association for Research in Vision and Ophthalmology
British Society for Immunology
New York Academy of Sciences
Royal Society for Tropical Medicine and Hygiene

V. ACADEMIC AND PROFESSIONAL HONORS

2000 President's Leadership Award, Chiron Corp.
1976-79 NIH Predoctoral Traineeship
1977 Fight for Sight Student Fellow
1980-81 Fight for Sight Postdoctoral Research Fellow

1982 NIH Individual Postdoctoral Fellowship
1983 Robert E. Shoemaker Research Award, Pennsylvania Academy of Ophthalmology and Otolaryngology
2000 President's Leadership Award, Chiron Research and Development

VI. ACADEMIC EXPERIENCE

A. Within the last five years

1988 - 1998 Adjunct Assistant Professor
Department of Ophthalmology
University of Pennsylvania School of Medicine
Philadelphia, Pennsylvania

B. Prior to the last 5 years

1983-88 Assistant Professor, Department of Ophthalmology
University of Pennsylvania School of Medicine
Philadelphia, Pennsylvania

1986-88 Graduate Group in Immunology
University of Pennsylvania
Philadelphia, Pennsylvania

1983-88 Graduate Group in Parasitology
University of Pennsylvania
Philadelphia, Pennsylvania

VII. EMPLOYMENT HISTORY

Position Title: Senior Director, Vaccine Research and Development
Department of immunology & Infectious Diseases
Chiron Research and Development
Chiron Corporation

Duration: July 2000-present

Brief Description of Significant Responsibilties:
Manage more than 20 Principal and Associate Scientists in research on HIV Vaccines and vaccine adjuvants and delivery. Direct Chiron HIV vaccine research and development program. Lead team responsible for externally financing HIV Vaccine R&D project; raised over $42 million of outside funds, mostly from NIH, since 1999. Direct clinical serology laboratory supporting Phase I-II studies of N. meningitidis group B vaccine. Provide research support for clinical studies of therapeutic hepatitis B vaccine and Interleukin-2 therapy of HIV. Direct basic research on serologic markers for immunity to Neisseria menigitidis group B. Direct basic research in cancer immunotherapy. Responsibilities include direction of basic research, direction of preclinical studies, budgeting, contribution to regulatory documentation, and presentations to outside regulatory groups such as the FDA Vaccines and Related Biologicals Advisory Committee. Chair of Institutional Animal Care and Use Committee for Chiron Corp, responsible for Emeryville and Seattle sites.

Position Title: Acting Vice President, Vaccine Research and Development
Chiron Research and Development
Chiron Corporation

Duration: February - July 2000
From departure of Vice President until new Vice President brought in from Chiron Siena, managed more than 35 Principal and Associate Scientists in research on HIV Vaccines, HCV Vaccines, DNA Vaccines, Vaccine Adjuvants and Delivery, and cell culture and reombinant protein production. Restructured Vaccines Research Department to achieve fiscal balance. Retained key personnel while reducing workforce by 15%. Managed internal and external HIV vaccine research and development activities. Directed clinical serology laboratory supporting Phase III studies of meningitis C conjugate vaccine (Menjugate®). Provided research support for clinical studies of therapeutic hepatitis B vaccine and Interleukin-2 therapy of HIV. Directed basic research on serologic markers for immunity to Neisseria menigitidis group B. Responsibilities included direction of basic research, direction of preclinical studies, budgeting, contribution to regulatory documentation, and presentations to outside regulatory groups such as the FDA Vaccines and Related Biologicals Advisory Committee. Chaired Institutional Animal Care and Use Committee for Chiron Corp.

Position Title: Director, Vaccine Adjuvants Research
Chiron Technologies
Chiron Corporation

Duration: 1998-present

Brief Description of Significant Responsibilties:
Manage more than 30 Principal and Associate Scientists in research on vaccine adjuvants, induction of cytotoxic T cells, DNA vaccines for HIV and HCV, cancer immunotherapy, gene therapy with MuLV-based viral vectors, and bacterial vacccines. Direct internal and external adjuvant research programs. Responsibilities include direction of basic research, direction of preclinical studies, budgeting, contribution to regulatory documentation, and presentations to outside regulatory groups such as the FDA Vaccines and Related Biologicals Advisory Committee. Beginning in September 1999, chaired Institutional Animal Care and Use Committee for Chiron Corp.

Position Title: Associate Director, Immunology
Dept. of Virus & Cell Biology
Merck Research Laboratories

Duration: 1994-98

Brief description of significant responsibilities:
Manage more than 10 Principal and Associate Scientists in basic research on DNA vaccines for influenza, HCV, and HPV, recombinant protein vaccines for Hepatitis B, vaccine adjuvants, and preclinical and clinical studies for Haemophilus influenzae type B and Streptococccus pneumoniae polysaccharide-protein conjugate vaccines. Studied cytotoxic T cell responses in nonhuman primates and cytokine responses in human subjects to experimental influenza DNA vaccines. Prepare regulatory documentation including preclinical sections of PLA's and Part III (Pharmaco-toxicological Documentation) of MAA's for bacterial vaccines and combination vaccines (Liquid PedvaxHIB®, COMVAX®, New Process Pneumovax 23®). Chaired Institutional Animal Care and Use Committee for West Point site.

Position Title: Research Fellow
Dept. of Virus & Cell Biology
Merck Research Laboratories

Duration: 1988-94

Brief Description of Significant Responsibilities:

Supervise up to 8 Principal and Associate Scientists in research on mechanisms of induction of cytotoxic T lymphocytes, including immunization with DNA, evaluation of adjuvants for clinical use in vaccines, development of analytical/serological assays for support of clinical vaccine programs (HPV, HIV, Influenza), preclinical development of bacterial vaccines, and preclinical development of influenza DNA vaccines. Direct research/licensing program in vaccine adjuvants and delivery systems.

1980-81 Postdoctoral Research Fellow, Department of Clinical Veterinary Medicine
University of Cambridge, Cambridge, England

1982 Postdoctoral Research Fellow, Department of Ophthalmology
Johns Hopkins University School of Medicine
Baltimore, Maryland

1983-88 Assistant Professor, Department of Ophthalmology
University of Pennsylvania School of Medicine
Philadelphia, Pennsylvania

IX. OTHER SKILLS, QUALITIES OR ACCOMPLISHMENTS

A. Membership on Peer Review Panels:

USAID Biotechnology/Immunology Panel	1988-1991
NIH/NIAID Review Committee for RFA NIH-NIAID-94-11, Basic Biology of Immune Responses for Vaccine Research	1994
NIH/NIAID Visual Sciences A Study Section, Ad hoc member	1997
NIH/NIDR Special Emphasis Panel on Oral Carcinoma	1997
USAID Schistosomiasis Vaccine Development Program Advisory Group, 4 year term beginning	1997
NIH/NIAID Vaccines Study Section, 3 year term beginning	1998

B. Editorial Boards:

Contributing Editor:

Autoimmunity	1988-
Current Eye Research	1987-
Cellular Immunology	1986-
Investigative Ophthalmology and Visual Science	1981-
Journal of Immunology	1994-

C. Meetings Organized

IBC First Annual Conference on Genetic Vaccines	1995
IBC Second Annual Conference on Genetic Vaccines	1996
IBC 4th Annual International Conference on Mucosal Immunization	1996
IBC Third Annual Conference on Genetic Vaccines	1997
IBC Fourth Annual Conference on Genetic Vaccines	1998
2nd Annual US Biotechnology Symposium	1999

D. Military Service:

Colonel, Medical Service Corps, U.S. Army Reserve

Blood Program Officer, Third United States Army (Forward), King Khalid Military City, Saudi Arabia, 12/22/90-4/1/91

X. PUBLICATIONS AND PATENTS

Donnelly, J.J., Rockey, J.H. and Soulsby, E.J.L.: Intraocular IgE antibody induced in guinea pigs with *Ascaris suum* larvae. Invest. Ophthalmol. Vis. Sci. 16: 976-981, 1977.

Rockey, J.H., Donnelly, J.J., Stromberg, B.E. and Soulsby, E.J.L.: Immunopathology of *Toxocara canis* and *Ascaris suum* infections of the eye: The role of the eosinophil. Invest. Ophthalmol. Vis. Sci. 18: 1172-1184, 1979.

Soulsby, E.J.L., Stromberg, B.E., Donnelly, J.J. and Rockey, J.H.: Intraocular immunoglobulin E induced by intravitreal infection with *Ascaris* and *Toxocara* spp. larvae. Ophthal. Res. 12: 45-53, 1980.

Rockey, J.H., Donnelly, J.J., Stromberg, B.E., Laties, A.M. and Soulsby, E.J.L.: Immunopathology of Ascarid infection of the eye: Role of IgE antibodies and mast cells. Arch. Ophthalmol. 99: 1831-1840, 1981.

Donnelly, J.J., Rockey, J.H., Bianco, A.E., and Soulsby, E.J.L.: Aqueous humor and serum IgE antibody in experimental ocular *Onchocerca* infection of guinea pigs. Ophthal. Res. 15: 61-67, 1983.

Rockey, J.H., John, T., Donnelly, J.J., McKenzie, D.F., Stromberg, B.E., and Soulsby, E.J.L.: In vitro interactions of eosinophils from ascarid-infected eyes with *A. suum* and *T. canis* larvae. Invest. Ophthalmol. Vis. Sci. 24: 1346-1357, 1983.

John, T., Donnelly, J.J. and Rockey, J.H.: Experimental ocular *Toxocara canis* and *Ascaris suum* infection: In vivo and in vitro study. Trans. Pa. Acad. Ophthalmol. Otolaryngol. 36: 131-137, 1983.

Attenburrow, D.P., Donnelly, J.J. and Soulsby, E.J.L.: Periodic ophthalmia (recurrent uveitis) of horses: An evaluation of the etiological role of microfilariae and the clinical management of the condition. Equine Vet. Journal 15: 48-56, 1983.

Donnelly, J.J., Rockey, J.H., Bianco, A.E. and Soulsby, E.J.L.: Ocular immunopathologic findings of experimental onchocerciasis. Arch. Ophthalmol. 102: 628-634, 1984.

Donnelly, J.J. and Prendergast, R.A.: Local production of Ia-inducing activity in experimental immunogenic uveitis. Cellular Immunology 86: 557-561, 1984.

Khatami, M., Donnelly, J.J., John, T. and Rockey, J.H.: Vernal conjunctivitis. Model studies on guinea pigs immunized topically with fluoresceinyl ovalbumin. Arch. Ophthalmol. 102: 1683-1688, 1984.

Lok, J.B., Pollack, R.J., Cupp, E.W., Bernardo, M.J., Donnelly, J.J., and Albiez, E.J.: Development of third-stage larvae of *Onchocerca volvulus* and *O. lienalis in vitro*. Tropenmedizin und Parasitologie 35: 209-212, 1984.

Donnelly, J.J., Vogel, S.N. and Prendergast, R.A., Down-regulation of Ia expression on macrophages by Sea Star Factor. Cellular Immunology 90: 408-415, 1985.

Rockey, J.H., Donnelly, J.J., John, T., Khatami, M., Schwartzman, R.M., Stromberg, B.E., Bianco, A.E. and Soulsby, E.J.L.: IgE antibodies in ocular immunopathology. Third International Symposium on the Immunology and Immunopathology of the Eye. Masson, New York, pp.199-202, 1985.

Khatami, M., Donnelly, J.J. and Rockey, J.H.: Induction and down-regulation of conjunctival Type-I hypersensitivity reactions in guinea pigs sensitized topically with fluoresceinyl ovalbumin. Ophthalmic Research 17: 139-147, 1985.

Donnelly, J.J., Li, W., Rockey, J.H. and Prendergast, R.A.: Induction of class II (Ia) alloantigen expression on corneal endothelium *in vivo* and *in vitro*. Invest. Ophthalmol. Vis. Sci. 26: 575-580, 1985.

Donnelly, J.J., Rockey, J.H., Taylor, H.R. and Soulsby, E.J.L.: Onchocerciasis: Experimental models of ocular disease. Reviews of Infectious Diseases 7: 820-825, 1985.

Donnelly, J.J., Taylor, H.R., Young, E.M., Khatami, M., Lok, J.B. and Rockey, J.H.: Experimental ocular onchocerciasis in cynomolgus monkeys. Invest. Ophthalmol. Vis. Sci. 27: 492-499, 1986.

Sakla, A.A., Donnelly, J.J., Lok, J.B., Khatami, M. and Rockey, J.H.: Punctate keratitis induced by subconjunctivally injected microfilariae of *Onchocerca lienalis*. Arch. Ophthalmol. 104: 894-898, 1986.

James, E.R., Smith, B. and Donnelly, J.J.: Invasion of the mouse eye by *Onchocerca* microfilarie. Trop. Med. and Parasitol. 37: 359-360, 1986.

Donnelly, J.J., Sakla, A.A., Hill, D.E., Lok, J.B., Khatami, M. and Rockey, J.H.: Effects of diethylcarbamazine citrate and anti-inflammatory drugs on experimental onchocercal punctate keratitis. Ophthalmic Research. 19: 129-136, 1987.

Lok, J.B., Pollack, R.J. and Donnelly, J.J.: Studies of the growth-regulating effects of Ivermectin on larval O. lienalis in vitro. J. Parasitol. 73: 80-84, 1987.

John, T., Barsky, H.J., Donnelly, J.J. and Rockey, J.H.: Retinal pigment epitheliopathy and neuroretinal degeneration in ascarid-infected eyes. Invest. Ophthalmol. Vis. Sci. 28: 1583-1598, 1987.

Donnelly, J.J., Xi, M.-S., Haldar, J.P., Hill, D.E., Lok, J.B., Khatami, M. and Rockey, J.H.: Autoantibody induced by experimental *Onchocerca* infection: Effects of different routes of administration of microfilariae and of treatment with diethylcarbamazine citrate and Ivermectin. Invest. Ophthalmol. Vis. Sci. 29: 827-831, 1988.

Donnelly, J.J., Chan, L.S., Xi, M.-S. and Rockey, J.H.: Effect of human corneal fibroblasts on lymphocyte proliferation *in vitro*. Exp. Eye Res. 47: 61-70, 1988.

Donnelly, J.J.: Autoantibody induced by experimental ocular *Onchocerca* infection: Identification and characterization of autoantigens. Autoimmunity. 1: 207-216, 1988.

Donnelly, J.J., Semba, R.D., Xi, M.-S., Young, E., Taylor, H.R. and Rockey, J.H.: Experimental vitreoretinal onchocerciasis: Role of IgG and IgE antibody and autoantibody and cell-mediated immunity. Tropical Medicine and Parasitology. 39: 111-116, 1988.

Pollack, R.J., Lok, J.B., and Donnelly, J.J.: Analysis of glutathione-enhanced differentiation by microfilariae of *Onchocerca lienalis* (Filaroidea: Onchocercidae) in vitro. J. Parasit. 74: 353-359, 1988.

Semba, R.D., Donnelly, J.J., Rockey, J.H., Lok, J.B., Sakla, A.A. and Taylor, H.R.: Experimental Onchocercal chorioretinitis. Invest. Ophthalmol. Vis. Sci. 29: 1642-1651, 1988.

Haldar, J.P., Khatami, M., Donnelly, J.J., Rockey, J.H.: Experimental allergic conjunctivitis: production of different isotypes of antibody by conjunctival associated lymphoid tissue in culture. Regional Immunology. 1: 92-98, 1988.

Khatami, M., Donnelly, J.J., Haldar, J.P. and Rockey, J.H.: Massive follicular lymphoid hyperplasia in experimental chronic recurrent allergic conjunctivitis. Archives of Ophthalmology. 107: 433-438, 1989.

Haldar, J.P., Donnelly, J.J., Khatami, M., Lok, J.B., Rockey, J.H.: Experimental ocular onchocerciasis: antibody production by conjunctival associated lymphoid tissue in culture. Tropical Medicine and Parasitology, 41: 234-240, 1990.

Donnelly, J.J., Orlin, S.E., Wei, Z.G., Raber, I.M., Rockey, J.H.: Class II alloantigeninduced on corneal endothelium: role in corneal allograft rejection. Invest. Invest. Ophthalmol. Vis. Sci. 31: 1315-1320, 1990.

Donnelly, J.J., Deck, R.R., Liu, M.A.: Immunogenicity of a *Haemophilus influenzae* polysaccharide-*Neisseria meningitidis* outer membrane protein complex conjugate vaccine. Journal of Immunology, 145: 3071-3079, 1990.

Lok, J.B., Morris, R.A., Sani, B.P., Shealy, Y.F., Donnelly, J.J., Synthetic and naturally occurring retinoids inhibit third-to-fourth stage larval development by *Onchocerca lienalis in vitro* Tropical Medicine and Parasitology 41: 169-173, 1990.

Semba, R.D., Donnelly, J.J., Young, E., Green, W.R., Scott, A.L., Taylor, H.R.: Experimental ocular onchocerciasis in cynomolgus monkeys. IV. Chorioretinitis elicited by Onchocerca microfilariae. Invest Ophthalmol Vis. Sci. 32:1499-1507, 1991.

Liu, M.A., Deck, R.R., Donnelly, J.J.: Use of the outer membrane protein complex of Neisseria meningitidis as a carrier for polysaccharide antigen. Polymer Preprints 32:217-218, 1991.

Liu, M.A., Friedman, A., Oliff, A.I., Tai, J., Martinez, D., Deck, R.R., Shieh, J. T.-C., Jenkins, T.D., Donnelly, J.J., Hawe, L.A.: A vaccine carrier derived from *Neisseria meningitidis*: with mitogenic activity for lymphocytes. Proc. Nat. Acad. Sci. (USA) 89:4633-37, 1992.

Ulmer, J.B., Burke, C.J., Shi, C., Friedman, A., Donnelly, J.J., Liu, M.A.: Pore formation and mitogenicity in red blood cells by the Class 2 protein of *Neisseria meningitidis*. J. Biol. Chem. 267:19266-19271, 1992.

Donnelly, J.J., Xi, M.-S., Rockey, J.H.: A soluble product of human corneal fibroblasts inhibits lymphocyte activation. Enhancement by Interferon-gamma. Exp. Eye Res. 56:157-165, 1993.

Ulmer, J.B., Donnelly, J.J., Parker, S.E., Rhodes, G.H., Felgner, P.L., Dwarki, V.J., Gromkowski, S.H., Deck, R.R., DeWitt, C.M., Friedman, A., Hawe, L.A., Leander, K.R., Martinez, D., Perry, H.C., Shiver, J.W., Montgomery, D.L., and Liu, M.A.: Heterologous protective immunity to influenza A by intramuscular injection of DNA encoding a conserved viral protein. Science 259:1745-1749, 1993.

Donnelly, J.J., Ulmer, J.B., Hawe, L.A., Friedman, A., Shi, X.-P., Leander, K.R., Shiver, J.W., Oliff, A.I., Martinez, D., Montgomery, D., and Liu, M.A.: Targeted delivery of peptide epitopes to MHC Class I by a modified *Pseudomonas* exotoxin. Proc. Nat. Acad. Sci. USA, 90:3530-3534, 1993.

Montgomery, D.L., Shiver, J.W., Leander, K.R., Perry, H.C., Friedman, A., Martinez, D., Ulmer, J.B., Donnelly, J.J., Liu, M.A. Heterologous and homologous protection against influenza A by DNA vaccination: Optimization of DNA vectors. DNA and Cell Biology 12:777-784, 1993.

Salerno, R.A., Mendelman, P.M., Donnelly, J.J., Fescharek, R., Ronneberger, H.: Haemophilus-influenzae-Typ-b-Konjugat-Impstoffe: Ergebnisse einer erweiterten Auswertung des Test auf anomale Toxizität. Monatsschr. Kinderheilkd. 142:364, 1994.

Ulmer, J.B., Donnelly, J.J., Liu, M.A.: Presentation of an exogenous antigen by major histocompatibility complex class I molecules. Eur. J. Immunol., 24:1590, 1994.

Ulmer, J.B., Deck, R.R., DeWitt, C.M., Friedman, A., Donnelly, J.J., Liu, M.A.: Protective immunity by intramuscular injection of low doses of influenza virus DNA vaccines. Vaccines, 12:1541-44, 1994.

Abrahamian, A., Xi, M.-S., Donnelly, J.J., Rockey, J.H.: Effect of interferon-gamma on the expression of transforming growth factor-beta in human corneal fibroblasts: Role in corneal immunoseclusion. J. Interferon Res. 15:323-330, 1995.

Jansen, K.U., Rosolowsky, M., Schultz, L.D., Markus, H.Z., Cook, J.C., Donnelly, J.J., Martinez, D., Ellis, R.W., Shaw, A.R.: Vaccination with yeast-expressed cottontail rabbit papillomavirus (CRPV) virus-like particles protects rabbits from CRPV-induced papilloma formation. Vaccine 13:1509-14, 1995.

Donnelly, J.J., Friedman, A., Martinez, D., Montgomery, D.L., Shiver, J.W., Motzel, S.L., Ulmer, J.B., Liu, M.A.: Preclinical efficacy of a prototype DNA vaccine: Enhanced protection against antigenic drift in influenza virus. Nature Medicine 1:583-587, 1995.

Donnelly, J.J., Ulmer, J.B., Liu, M.A.: Protective efficacy of intramuscular immunization with naked DNA. Ann. N.Y. Acad. Sci. 772:40-6, 1995.

Donnelly, J.J., Martinez, D., Jansen, K.,U., Ellis, R.W., Montgomery, D.L. Liu, M.A.: Protection against papillomavirus with a polynucleotide vaccine. J. Inf. Dis. 713:314-20, 1996.

Anderson, E.L., Kennedy, D.J., Geldmacher, K.M., Donnelly, J.J., Mendelman, P.M.: Immunogenicity of heptavalent pneumococcal conjugate vaccine in infants. J. Pediatrics 128:649-563, 1996.

Ulmer, J.B., Deck, R.R., DeWitt, C.M., Donnelly, J.J., Liu, M.A.: Generation of MHC Class I-restricted cytotoxic T lymphocytes by expression of a viral protein in muscle cells: Antigen presentation by non-muscle cells. Immunology 1996, 89:59-67.

Fu, T.-M., Friedman A., Ulmer, J.B., Liu, M.A., Donnelly, J.J.: Protective cellular immunity: Cytotoxic T-lymphocyte responses against dominant and recessive epitopes of influenza virus nucleoprotein induced by DNA immunization. J. Virology, 1997, 71:2715-2721.

Mendelman, P.M., Feeley, L., Bird, S., Staub, T., Matthews, H., Del Beccaro, M., Overturf, G., Lee, A., Ellis, R., Staub, J., Szymanski, S., Donnelly, J.J., Hennessey, J.P., Kniskern, P.: Immunogenicity and safety of *Haemophilus influenzae* type b polysaccharide-*Neisseria meningitidis* conjugate vaccine in 7.5 μg liquid formulation: a comparison of three lots with the 15.0 μg lyophilized formulation. Vaccine 1997, 15:775-761.

Ulmer, J.B., Deck, R.R., DeWitt, C.M., Fu, T.-M., Donnelly, J.J., Caulfield, M.J., Liu, M.A.: Expression of a viral protein by muscle cells in vivo induces protective cell-mediated immunity. Vaccine 1997, 15:839-841.

Donnelly, J.J., Friedman, A., Ulmer, J.B., Liu, M.A.: Further protection against antigenic drift of influenza virus in a ferret model by DNA vaccination. Vaccine 1997, 15:865-868.

Liu, M.A., McClements, W.L., Ulmer, J.B., Shiver, J.W., Donnelly, J.J.: Immunization of non-human primates with DNA vaccines. Vaccine 1997, 15:909-912.

Fu, T.-M., Ulmer, J.B., Caulfield, M.J., Deck, R.R., Friedman A., Wang, S., Liu, X., Donnelly, J.J., Liu, M.A.: Priming of cytotoxic T lymphocytes by DNA vaccines: Requirement for professional antigen-presenting cells and evidence for antigen transfer from myocytes. Molecular Medicine 1997, 3:362-371.

Ulmer, J.B., Fu, T.M., Deck, R.R., Friedman, A., Guan, L., DeWitt, C.M., Liu, X., Wang S., Liu, M.A., Donnelly, J.J., Caulfield, M.A.: Protective CD4+ and CD8+ T cells against influenza virus induced by vaccination with nucleoprotein DNA. J. Virology 72: 5648-5653, 1998.

Bender, B.S., Ulmer, J.B., DeWitt, C.M., Cottey, R., Taylor, S.F., Ward, A., Friedman, A., Liu, M., Donnelly, J.J.: Immunogenicity and efficacy of DNA vaccines encoding influenza A proteins in aged mice. Vaccine, 16:1748-55, 1998.

Fu, T.-M., Guan, L., Friedman, A., Ulmer, J.B., Liu, M.A., Donnelly, J.J.: Induction of MHC Class I-restricted CTL Response by DNA immunization with ubiquitin-influenza virus nucleoprotein fusion antigens. Vaccine, 16:1711-7, 1998.

McNeely, T.B., Staub, J.M., Rusk, C.M., Blum, M.J., Donnelly, J.J.: Antibody responses to capsular polysaccharide backbone and O-acetate side groups of *Streptococcus pneumoniae* type 9V. Infect. Immun. 66:3705-10, 1998.

Fu, T.-M., Guan, L., Friedman, A., Ulmer, J.B., Liu, M.A., Donnelly, J.J.: Dose- dependence of CTL precursor frequency induced by a DNA vaccine and correlation with protective immunity against influenza virus challenge. J. Immunol 162:4163-4170, 1999.

Ulmer, J.B., DeWitt, C.M., Chastain, M., Friedman, A., Donnelly, J.J., McClements, W.L., Caulfield, M.J., Bohannon, K.E., Volkin, D.B., Evans, R.K.: Enhancement of DNA Vaccine Potency Using Conventional Aluminum Adjuvants. Vaccine, 18:18-28, 2000.

McNeely, T.B., Liu, X., Bringman, T., Donnelly, J.J.: Effect of individual conjugate dose on immunogenicity of multivalent pneumococcal polysaccharide-*N. meningitidis* outer membrane protein complex conjugate vaccine. Vaccine, 18:2808-16, 2000.

Dupuis, M., Denis-Mize K., Woo C., Goldbeck, C., Selby, M.J., Chen, M., Otten G.R., Ulmer, J.B., Donnelly, J.J., Ott, G., and McDonald, D.M.: Distribution of DNA vaccines determines their immunogenicity after intramuscular injection in mice. J. Immunol. 165:2850-2858, 2000.

Denis-Mize, K.S., Dupuis M., MacKichan, M.L., Singh, M., Doe, B., O'Hagan, D., Ulmer, J.B., Donnelly, J.J., McDonald, D.M., and Ott, G.: Plasmid DNA adsorbed onto caitonic micropraticles mediates target gene expression and antigen presentation by dendritic cells. Gene Therapy, in press.

Ugozzoli, M., Santos, G., Donnelly, J.J., and O'Hagan, D: The potency of a genetically toxoided mucosal adjuvant derived from the heat labile enterotoxin of Escherichia coli (LTK63) is not adversely affected by the presence of pre-existing immunity to the adjuvant. J. Infec. Dis. 183:351-4, 2001.

Singh M., Ott G, Kazzaz J, Ugozzoli M, Briones M, Donnelly J, O'Hagan DT. Cationic microparticles are an effective delivery system for immune stimulatory CpG DNA. Pharm. Res., 18:1476-9, 2001.

Santos GF, Deck RR, Donnelly JJ, Blackwelder W, and Granoff DM. Importance of complement source when measuring meningococcal bactericidal titers as a surrogate of protective efficacy. Clin Diagn. Lab Immunol. 8:616-23, 2001.

Srivastava, I.K., L. Stamatatos, A. Fong, H. Legg, E. Kan, S. Coates, L. Leung, M. Wininger, A.R. Tipton, J. Donnelly, J.B. Ulmer, and S.W. Barnett. Purification and characterization of oligomeric glycoprotein from a primary R5 subtype B human immunodeficiency virus for vaccine applications. J. Virology. 76:2835-47, 2002.

Anderson E.L., Frey S.L., Geldmacher K., Radley D., Lee A., Donnelly, J.J., Mendelman, P.M., Dargan J.M., Kaplan K.M. Safety, tolerability and immunogenicity of low dose Haemophilus influenzae type b conjugated to the outer membrane protein complex of Neisseria meningitidis group B. Ped. Inf. Dis. J. 21:350-2, 2002.

PATENTS

Donnelly, J.J., Liu, M.A., Martinez, D., and Montgomery, D.L. Polynucleotide vaccine for papillomavirus. US Patent 5,866,553. Feb. 2, 1999.

INVITED REVIEWS and BOOK CHAPTERS

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Donnelly, J.J., Li, W., Chan, L. and Rockey, J.H.: Induction of corneal class II (Ia) alloantigen expression in immunogenic uveitis. In, Modern Trends in the Immunology and Immunopathology of the Eye, ed., Secchi, A.G., Fregona, I.A. Masson, Milan, pp. 242-248, 1989.

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DISSERTATION

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EXHIBIT B

Priming of both B cell anti-HIV envelope responses by vaccination is essential for the long lasting containment of viral replication in macaques infected with an R5-tropic SHIV isolate.

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ABSTRACT

Vaccines eliciting cellular anti-HIV/SIV responses but no neutralizing antibodies may more effectively control infection of primates by X4- or X4R5-tropic viruses than R5-tropic viruses, which are preferentially transmitted in humans. To determine whether long lasting control of R5-tropic virus replication is achieved when the vaccine elicits neutralizing antibodies, we immunized macaques with the HIV envelope using the 'DNA-prime / protein-boost' methodology and depleted them of their CD8+ cells prior to challenge with the R5-tropic, $SHIV_{SF162P4}$ virus. Although the presence in the vaccinated animals of neutralizing antibodies at the time of challenge significantly reduced plasma viremia during acute-infection, the extent to which viral replication was controlled during the chronic phase of infection was dependent on the rapidity with which potent anamnestic neutralizing antibody responses were developed following challenge. Thus, even though the control animals developed anti-viral responses they did so more slowly than the vaccinated animals, and as a result the former, but not the latter, animals remained viremic, with decreasing CD4+ T cell numbers during the period of observation and some died from AIDS. Our studies emphasize the importance of priming by vaccination the B cell responses against the HIV envelope to effectively control HIV-replication during chronic infection.

INTRODUCTION

Vaccination methodologies that elicit cellular anti-HIV/SIV responses in the absence of measurable neutralizing antibodies, offer partial protection to macaques challenged with the dual-tropic (R5X4) pathogenic SHIV89.6P virus [1-3]. The benefit offered by such vaccines is primarily evident during the chronic phase of infection, when a significant reduction in plasma viral load levels is recorded in the vaccinated, as compared to unvaccinated, animals. Importantly, most vaccinated animals are protected from the rapid depletion of their CD4+ T cells and the rapid progression to disease, hallmarks of SHIV89.6P-infection [4]. However, the emergence of escape mutants has been recorded in some of these animals, which eventually developed AIDS [5]. In addition, priming by vaccination of cellular anti-viral responses alone may not be an effective way to control infection by pathogenic R5-tropic viruses, such as SIVmac239 [6].

It is well established that passive administration of neutralizing antibodies to macaques prior to their exposure to virus (even to R5-tropic viruses) results in sterilizing immunity, but only when the neutralizing antibody titers at the time of viral exposure are high [7-17]. Whether the various vaccination methodologies that are currently evaluated will be able to elicit and sustain for extended periods of time neutralizing antibodies at titers capable of offering sterilizing immunity, is unknown. It is also not well established whether vaccine-elicited neutralizing antibodies at titers that are suboptimal for sterilizing immunity, would offer any benefit to the vaccinee. Finally it is unknown whether the presence of memory B cells with anti-HIV envelope specificity will be beneficial to a vaccinated, but infected host.

To address the above issues, we immunized rhesus macaques with the HIV envelope derived from the R5-tropic SF162 and the related SF162ΔV2 viruses using the 'DNA-prime plus protein-boost' vaccination methodology. To reduce the impact of the cellular anti-HIV responses during

acute infection, and to primarily evaluate the protective role of antibodies during this period, we transiently depleted the CD8+ cells from these animals prior to their exposure to the R5-tropic, highly replication-competent $SHIV_{SF162P4}$ virus [18,19]. $SHIV_{SF162P4}$-infection of rhesus macaques is characterized by high plasma viral load levels (10^7-10^8 viral RNA copies per ml blood) during the acute phase of infection; varying levels of plasma viremia during the chronic phase of infection; a gradual depletion of CD4+ T lymphocytes; and a gradual progression to disease. We report that although the titers of neutralizing antibodies elicited during vaccination were not sufficient to offer sterilizing immunity, they significantly reduced the viral load during the acute phase of infection. In addition, shortly after exposure to virus the vaccinated animals rapidly developed high titers of *de novo* neutralizing antibody responses, which in combination with CTL responses effectively controlled viral replication during the chronic phase of infection. The control animals were slower in developing anti-viral responses, but such responses were present in these animals during chronic infection. Despite their ability to mount anti-viral responses, the control animals were not capable of effectively controlling viral replication and some succumbed to AIDS.

Our studies underscore the crucial role that neutralizing antibodies play in controlling replication of R5-tropic SHIVs both during the acute and chronic phases of infection. They strongly suggest that vaccines against HIV should not only prime for cellular responses against multiple viral antigens, but also for anti-HIV envelope B cell responses in order to limit viral replication during the early stages of infection and allow the development of *de novo* antiviral responses capable of controlling infection for extended periods of time.

RESULTS

Pilot vaccination studies

We previously reported that two rhesus macaques (H445 and J408) immunized with the gp140 form of the HIV envelope derived from the SF162ΔV2 virus [18] (ΔV2gp140), using the 'DNA-prime plus protein-boost' immunization methodology, developed strong neutralizing antibodies that, in the absence of CD8+ cells, reduced plasma viral load levels during acute infection by the highly-replication competent, R5-using $SHIV_{SF162P4}$ virus [18]. Here we report that both animals remained negative for plasma viral RNA for over two years (Fig. 1*a*), with stable numbers of CD4+ and CD8+ T lymphocytes (Fig. 1*b* and 1*c*, respectively), no signs of disease and no evidence for the emergence of escape viruses. During this period, the anti-HIV envelope antibody titers remained stable (Fig. 1*d*). CTL anti-viral responses against the SIV gag (primarily) and the HIV env proteins were measurable during chronic infection in these animals (Fig. 1*e*).

To evaluate in more detail the role of CTLs and neutralizing antibodies in controlling viral replication during chronic infection, 886 days post-challenge we administered the human/mouse hybrid anti-CD8 MAb cm T807 [20,21] in these animals. The CD8+ cell numbers were thus artificially depleted for 7 days in animals H445 and 14 days in animal J408 (Fig 1 *c*). The observed duration of CD8+ cell-depletion is shorter than what is usually achieved with this anti-CD8 MAb [20,21]. We believe that this is due to the fact that these two animals had been already infused with the mouse antiCD8 MAb, OKT8F, [18] and thus had been sensitized against mouse IgG. Concomitant with this reduction in CD8+ cell numbers, a reduction in cellular-mediated activity (Fig. 1*e*) and a burst in viremia (Fig.1*a*) (13,084 and 115,475 RNA copies per ml in animal H445 and J408, respectively) was recorded in the periphery. The fact that weak SIV gag-specific cellular responses were recorded in PBMC collected at the time of CD8+ cell-depletion (for example at 3 days post

cm T807 administration), suggests the presence of CD4+ cells with anti-viral activity in these animals. Once the CD8+ T cells re-appeared in the periphery, an increase in cellular-mediated anti-viral activity was evident and a concomitant reduction in plasma viremia to undetectable levels was once again recorded. In parallel to the increase in plasma viremia upon CD8+ cell-depletion, a rapid increase in the anti-HIV envelope antibody titers was recorded in both animals (Fig 1*d*). These observations suggest that both the cellular and humoral arms of the immune system are functional in these two animals and that both types of immune responses most likely contribute in the effective long-term control of viral replication.

In contrast, the two control animals of this group (AT54 and A141) had sustained viremia throughout the entire period of observation. One of them (animal AT54) had very high levels plasma viral load during and following acute infection; decreasing numbers of CD4+ T lymphocytes; and died from SAIDS within 16 weeks post-challenge (Fig 1*a-d*) [18]. The second control animal (A141) had high levels of plasma viremia during acute infection and remained viremic during chronic infection (Fig 1*a*), with stable CD4+ and CD8+ T cell numbers in the blood for the first 520 days following infection (Fig 1*b and c*, respectively). Subsequently, both CD4+ and CD8+ T cell numbers decreased. During the entire period of observation the anti-HIV envelope binding antibody titers gradually increased (Fig 1*d*). This animal was euthanized at day 670 post-infection because of health complications unrelated to $SHIV_{SF162P4}$-infection. However, at necropsy this animal had appreciable viremia in the spleen (143,000 RNA copies per mg), inguinal (38,450 RNA copies per mg), auxillary (16,000 RNA copies per mg) and mesenteric (722 RNA copies per mg) lymph nodes. While CD8+ T lymphocytes were present in these tissues at necropsy, CD4+ T lymphocytes were undetectable (data not shown).

Immunization of a second group of animals

To confirm the above observations and to examine in more detail the protective role of vaccine-elicited neutralizing antibodies during the acute phase of infection, a second group of animals (K863, P655, I708 and N473) was similarly immunized with the envelope protein derived from the SF162ΔV2 (as the first group of animals) or the related SF162 viruses [22] (SF162gp140 and ΔV2gp140, respectively). Potent binding-antibody responses were generated in all four animals following the 'booster' immunization with the CHO-produced soluble oligomeric gp140 envelope proteins (Fig. 2*a*), similar to what we recorded with the first group of animals [18]. However, the titer of binding antibodies did not always correlate with the titer of neutralizing antibodies against the challenge virus, $SHIV_{SF162P4}$ (Fig. 2*b*), as we previously reported for the first group [18]. In fact, out of the six immunized animals in these two groups, only two (J408, from the first group, and N473, from the second group) developed neutralizing antibody responses, potent enough to neutralize the challenge $SHIV_{SF162P4}$ virus by 90% at a serum dilution of 1:5, during *in vitro* neutralization using activated human PBMC as target cells (Fig. 2*b* and [18]). At the end of the immunization protocol, weak anti-HIV envelope cellular mediated responses were recorded in three out of the four vaccinated animals (Fig. 2*c*).

Depletion of CD8+ cells prior to viral-challenge: Despite our inability to generate stronger anti-viral cellular mediated responses, we decided to deplete the CD8+ cells from these animals prior to their challenge with virus to eliminate any potential involvement of these cells during the early stages of infection and to better appreciate the benefits offered by the vaccine-elicited neutralizing antibodies during this period of time. CD8+ cell-depletion was achieved as previously reported for the vaccinated animals of the first group [18], by administrating the anti-CD8 MAb

OKT8F (Fig. 3). An isotype control MAb, OKT3, was administered to the control animals M844 and C640. OKT8F-administration resulted in the transient depletion of CD8+ cells from the periphery, which lasted between 9 (animal P655, Fig. 3*d*) and 14 days (animal K863, Fig. 3*b*). During the same period, the CD8+ cells were also depleted from the axillary and inguinal lymph nodes examined (data not shown). Cellular-mediated anti-HIV envelope (or SIV gag) responses were not detectable during this period in the periphery (see below). Subsequently, CD8+ T lymphocytes gradually re-emerged in the periphery and the lymph nodes of the vaccinated animals (Fig. 3 and data not shown).

Inverse correlation between acute plasma viremia levels and titers of vaccine-elicited neutralizing antibodies: Both the vaccinated and the control animals of the second group became infected upon their intravenous exposure to $SHIV_{SF162P4}$ (Fig. 4*a*). Peak levels of plasma viremia were recorded approximately 12-15 days post-challenge in all animals. Although the titers of vaccine-elicited neutralizing antibodies were not sufficient to offer sterilizing immunity, the differences in the peak viral load levels during acute infection between the vaccinated (Mean peak viral load of 1,794,000 and standard deviation of 891,900) and control animals (Mean peak viral load of 9,784,000 and standard deviation of 178,400) is statistically significant (P: 0.004, R^2: 0.8985) (Fig. 4*b*). Importantly, animal N473 with the strongest neutralizing antibody responses at the day of challenge (Fig 2*b*) had the lowest peak viremia levels during acute infection (7,180 RNA copies per ml). These observations are in agreement with those made with the first group of animals, where animal J408 with the highest neutralizing antibody titers at the time of challenge, had lower plasma viremia following infection (8,030 RNA copies per ml) than the second immunized animal, H445, which had weaker neutralizing antibody responses (Fig 1*a*) and [18].

Development of anamnestic anti-viral responses and effective long-term control of infection

All the vaccinated animals developed potent anamnestic binding anti-HIV envelope antibody responses during the first two weeks post-challenge (Fig. 4*c*). The vaccinated animals with the lower neutralizing antibody titers at the day of challenge (animals I708, K863 and P655) developed stronger anamnestic binding-antibody responses than the vaccinated animal with the strongest neutralizing antibody responses at the day of challenge (animal N473). In parallel, the titers of neutralizing antibodies increased in all vaccinated animals (Fig. 4*d*). The emergence in the vaccinated animals of strong neutralizing antibody responses coincided with the recorded decrease in plasma viremia during acute infection. All the vaccinated animals were able to effectively control viral replication during the chronic phase of infection (Fig. 4*a*), so that with the exception of animal P655, virus has remained undetectable in the periphery of these animals for almost two years following infection.

In the control animals, the appearance of binding and neutralizing antibodies (Fig. 1*c,d*) was delayed by one to two weeks as compared to the vaccinated animals and occurred following the initial reduction in plasma viremia during acute infection (Fig 1*a*). This reduction is therefore most likely due to the emergence of cellular-mediated anti-viral responses (Fig. 5). At the earlier time point tested (day 6 post-infection) the anti-viral cellular responses were undetectable, both in the vaccinated (which at the time lacked CD8+ cells) and the control animals (Fig. 5). CTL responses were, however, recorded in all animals at later time points. No difference in the rate at which cellular anti-viral responses were generated in the vaccinated and control animals was evident. During the period of observation, these responses were maintained in some, but not all animals.

The anti-SIV gag responses (Fig. 5*a*) were more potent than the anti-HIV env responses (Fig. 5*b*) in all animals and at all time points tested. PBMC from all animals were susceptible to PHA-mediated stimulation throughout course of infection (Fig. 5*c*). The control animal C640 with the highest set-point plasma viral load (Fig. 4*a*) had the strongest cellular anti-viral responses, in accordance with recent human data during chronic HIV-infection [23]. However, despite the fact that this animal developed both neutralizing antibodies and potent cellular-mediated anti-viral responses, it failed to eliminate the virus from the periphery (Fig 4*a*).

We have monitored this second group of $SHIV_{SF162P4}$-infected animals for almost two years. All the vaccinated animals have stable CD4+ T cell numbers (Fig. 4*e*) and, with the exception of animal P655 (10^3 to 10^4 RNA copies per ml), they remain plasma RNA-negative. The control animal C640 remained plasma RNA positive (approximate 10^5 RNA copies per ml of plasma) for the duration of observations, with decreasing CD4+ T cells in the periphery (Fig. 4*e*) and eventually developed sAIDS. In contrast, the control animal M844 has remained plasma RNA negative and with stable CD4+ T lymphocyte numbers during the same period of observation, even though during acute infection this animal had similar high viremia as animal C640.

DISCUSSION

The ultimate goal of a vaccine against HIV is to prevent infection. To this end, an effective vaccine must elicit the generation of very high titers of neutralizing antibodies [10,17] especially against R5-tropic viruses which are primarily transmitted in humans[24-31]. Our challenge studies were conducted with the R5-tropic, highly replication-competent $SHIV_{SF162P4}$ virus. This virus was chosen because in contrast to all the other known SHIVs, is R5-tropic **like the HIV isolates responsible for transmission in humans (refs).** In addition, $SHIV_{SF162P4}$-infection of macaques

better mimics infection of humans by HIV, as compared to infection of macaques with highly pathogenic X4- or X4R5-tropic SHIVs, which induce an unusually rapid-depletion of CD4+ T lymphocytes and a very rapid progression to disease and to death [4,32,33]. The envelope $SHIV_{SF162P4}$ is closely related to the envelope immunogens SF162gp140 and SF162ΔV2gp140 immunogens used here. As a result we did not record differences between the SF162 and SF162ΔV2-immunized animals in their ability to control infection by $SHIV_{SF162P4}$. Future studies will establish which immunogen is more effective in eliciting antibodies capable of protecting from heterologous viral challenge. However, the current studies allowed us to establish the levels of neutralizing antibodies required to be elicited by the DNA-prime plus protein-boost vaccination methodology, in order to record a significant reduction in plasma viremia during infection upon heterologous viral challenge.

During our vaccination studies we were not able to elicit titers of neutralizing antibodies capable of offering sterilizing immunity to $SHIV_{SF162P4}$-challenge by the intravenous route. However, the vaccinated animals had significantly lower peak plasma viremia titers as compared to control animals, despite the fact that we depleted the CD8+ cells from the former and not from the latter animals prior to viral-exposure. The vaccinated animals with the strongest neutralizing antibody responses at the day of challenge had up to $4\log_{10}$ lower levels of peak plasma viremia as compare to the controls. Such a dramatic reduction in plasma viral load levels during acute infection is not usually observed in animals immunized with vaccines that elicit primarily (or exclusively) cellular-mediated, but not measurable neutralizing antibody, responses against the challenge virus [1-3,6]. Therefore the significant reduction in viremia during acute infection recorded is primarily due to the neutralizing antibodies elicited during vaccination. However, it is also possible that vaccine-elicited antibodies may also have contributed to the elimination of viral

particles via non-neutralization-mediated mechanisms. Additionally, cellular responses that are not mediated by CD8+ cells could be elicited during DNA-immunization and participate in the early control of viral replication. Irrespective of the extent to which viral replication was reduced during acute infection, all vaccinated animals rapidly developed *de novo* binding and neutralizing antibodies. Such responses were also generated in the control animals but their emergence was delayed as compared to the vaccinated animals. In contrast, cellular anti-viral responses emerged at the same rate in the vaccinated and control animals. We assume that if the CD8+ cells were not artificially depleted from the vaccinated animals prior to viral challenge, cellular anti-viral responses would have emerged earlier in these animals.

Although most of the vaccinated animals remained aviremic during chronic infection and all have stable CD4+ T cells numbers, most control animals remain viremic and some had decreasing numbers of CD4+ T cell numbers in the periphery and eventually developed AIDS.

In a recent study, macaques immunized with a DNA-prime plus vaccinia-boost immunization regiment developed cellular, but not neutralizing antibody, responses against the challenge R5-tropic SIVmac239 virus [6]. During acute infection, these animals had reduced viral load levels, as compared to controls, and developed robust cellular mediated antiviral responses, but not neutralizing antibodies. Despite the development of such responses, these animals progressed to disease as rapidly as the control animals. One of the differences between that study and ours is that in our case, neutralizing antibodies were present at the time of challenge and their titer rapidly increased following challenge. Thus, vaccination methodologies that prime both B and T cell responses maybe more effective in controlling infection by highly replication-competent R5-tropic viruses, that those primarily, or exclusively, priming the cellular arm of the immune system. Although our challenge studies were conducted with a virus that expresses an envelope that is

closely related to our immunogens, our studies suggest that vaccines the elicit even low titers of neutralizing antibodies against heterologous HIV isolates, could be very beneficial.

Overall our data indicate that vaccine-elicited suboptimal titers of neutralizing antibody against HIV will be able to limit viral replication during the early stages of infection, even in the absence of CD8+ cell-mediated anti-viral responses. The ability however of the immune system to control viral replication during the chronic phase of infection is not dependent on the extent to which viral replication is slowed down during acute infection, but on the rapidity with which *de novo* antibody and cellular antiviral responses are generated by the infected host. Thus, priming of B cell mediated anti-HIV responses by vaccination is crucial for the effective control of HIV infection.

METHODS

Vaccines and vaccination methodology: A detailed description of the vaccination methodology, as well as the generation and production of the various immunogens, was reported earlier [34]. Briefly, the animals received three monthly immunizations with DNA vectors expressing the gp140 envelope form derived from the SF162 or SF162ΔV2 viruses [22]. 5-10 months later they were immunized a fourth time with DNA and at the same time with the corresponding recombinant gp140 proteins produced in CHO cells, purified as oligomers and adjuvanted in MF-59C (Chiron Co. Emeryville, CA). The control animal M844 received three administrations of the 'empty' DNA vector and the MF-59C adjuvant (mock-immunization).

Determination of CD3+, CD4+ and CD8+ T cell-numbers in the periphery and lymph nodes: Absolute CD3+, CD4+ and CD8+ T lymphocyte numbers were measured on a Coulter Epics XL.MCL Flow-cytometer using anti-CD3-FITC (clone SP34, BD, San Diego, CA), anti-

CD4-PE (clone M-T477, BD, San Diego, CA) and anti-CD8-PC5 (clone B9.11, Immunotech, Beckman Coulter). Appropriate anti-mouse Ig isotypes were used as negative controls.

Antibody-responses: a) Binding antibodies: Titers were determined throughout the immunization protocol and following viral-challenge as previously described [34]. Briefly, purified soluble oligomeric ΔV2gp140 and SF162gp140 proteins were adsorbed onto Immulon 2HB 96 well plates (ThermoLabsystems, Franklin, MA) by an overnight incubation at room temperature. Serially-diluted heat-inactivated (56^0C for 35 minutes) sera collected from the immunized animals were added to the wells (0.1 ml per well) for one hour at 37^0C. Pre-immunization sera were used as negative controls. Envelope-bound serum antibodies were detected with the use of goat anti-human IgG coupled to alkaline phosphatase antibodies (Zymed Immunochemicals, South San Francisco, CA) and the appropriate substrate/amplifier combination (Dako CO. Carpinteria, CA). **b) Neutralizing antibodies:** Neutralization assays were performed using as target cells human PBMC that were activated for three days with PHA (Sigma, 3 μg/ml) as we previously described [34]. $SHIV_{SF162P4}$ (100 $TCID_{50}$ in 50 μl of complete RPMI media containing 40 U/ml of IL-2 (Hoffmann-La Roche)) was pre-incubated in triplicate with an equal volume of serially diluted heat-inactivated pre-immunization, post-immunization, or post-SHIV-challenge sera for one hour at 37^0C, in 96 well U-bottom plates (Corning). Pre-immunization sera served as controls for non-specific neutralization. To each well, 0.1 ml of complete media containing 0.4×10^6 PHA-activated human PBMC was added. Following a 24-hour incubation at 37^0C, half the volume of each well was replaced with complete RPMI media. Following centrifugation of the plates (5 minutes at 2,000 rpm), half the volume of each well was again replaced with fresh media. The concentration of p27 antigen in the supernatant was determined with a commercially available kit (ZeptoMetrix Corporation, Buffalo, NY). The percent inhibition of infection at a given dilution of

sera collected post-vaccination or post-infection sera was calculated taking into account the non-specific neutralization recorded with sera collected prior to the initiation of immunization [34].

CD8+ cell-depletion: a) During acute infection: The immunized animals were infused with the mouse anti-CD8 MAb OKT8F as previously described [35]. Control animals M844 and C640, were similarly treated with an unrelated MAb (OKT3). CD8-depletion was evaluated using the anti-CD8 MAb OKTA-FITC that recognizes a different epitope than that recognized by OKT8F (data not shown). **b) During chronic infection:** Depletion of CD8+ cells during the chronic phase of infection was achieved by infusing the anti-CD8 MAb, cm T807, as previously reported [20].

$SHIV_{SF162P4}$-challenge: Seven weeks following the end of the immunization and one day following the last administration of the anti-CD8 antibody OKT8F, the animals were challenged by an intravenous administration of 100 $TCID_{50}$ of a cell-free stock of the $SHIV_{SF162P4}$ virus [19].

Viral load measurements: The SIV bDNA assay (Bayer Reference Testing Laboratory, Emeryville, CA) was employed to quantify plasma SIV RNA copy numbers. The lower quantitation limit of this assay is 500 SIV RNA copies per ml.

ELISPOT: Cryopreserved PBMC were plated at a density of 2×10^5 per well onto wells of a 96-well flat-bottomed plate that were previously coated with anti-IFN-γ antibody (Ucytech # CT 126). Pooled peptides (15-mers with an 11 amino acid overlap) derived from the homologous SIVmac239 Gag or the related $SHIV_{SF162P3}$ Env (NIAID, Reference and Reagent Program) were then added into the wells, at a final concentration of 1 µg/ml for each individual peptide. As a positive control, Staphylococcus Enterotoxin B (SEB) was added to the wells at a final concentration of 5 µg/ml. As negative control, PBMC were plated in medium alone. The cells were incubated for 24 hrs at 37°C and processed according to manufacturer's specifications using

the Monkey IFN-γ ELISPOT Kit (Ucytech). Only ELISPOT counts of twice the background were considered significant (over 20 counts per well).

ACKNOWLEDGMENTS

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FIGURE LEGENDS

Fig. 1 Long-term suppression of $SHIV_{SF162P4}$ replication in vaccinated macaques. Plasma viral load (***a***), plasma CD4+ T cell numbers (***b***), plasma CD8+ T cell numbers and (***c***) evolution of binding

anti-HIV envelope antibody responses (***d***), in control (A141, ▼, and AT54, ♦) and vaccinated (J408, ◦, and H445, ■) animals following intravenous challenge with $SHIV_{SF162P4}$. Black arrows indicate the time of administration of the anti-CD8 MAb OKT8F prior to viral challenge and white arrows indicate the administration of the anti-CD8 MAb cm T807 during the chronic phase of infection. (†) Indicates the time of death (***e***). Anti-SHIV CTL responses during chronic infection were determined with the ELSIPOT assay using PBMC collected at the indicated time points following viral-challenge. Numbers in parenthesis indicate the time of PBMC-collection in respect to administration of the anti-CD8 MAb cm T807. IFN-γ production following stimulation with peptides derived from the HIV env (green) or the SIVgag (blue). Responses obtained with SEB (red) are also shown.

Fig. 2 Development of antibody responses during 'DNA-prime plus protein-boost' vaccination with the SF162gp140 and ΔV2gp140 proteins. ***a***, Generation of binding antibodies against the soluble oligomeric gp140 HIV envelope proteins during three DNA administrations (black arrows) and following a fourth DNA administration in combination with the purified recombinant gp140 proteins (white arrow). Animals P655 (□) and N472 (○) were immunized with the SF162gp140 envelope immunogen and animals I708 (▼) and K863 (♦) with the related ΔV2gp140 envelope immunogen; ***b***, neutralizing potency of serum antibodies at the day of viral-challenge against the challenge $SHIV_{SF162P4}$ virus. Horizontal dotted lines indicate the 90, 70 and 50% levels of neutralization; ***c***, cellular mediated responses at the end of the immunization schedule (2 weeks post protein-boosting).

Fig. 3 Transient depletion of CD8+ cells from the vaccinated macaques. The vaccinated animals I708 (***a***), K863 (***b***), N472 (***c***) and P655 (***d***) were infused with the anti-CD8 MAb OKT8F, while the control animals M844 (***e***) and C640 (***f***) were infused with the isotype control MAb OKT3. Arrows indicate the time of OKT8F-infusion and the dotted line indicates the time of $SHIV_{SF162P4}$-challenge. ◊, CD8+ T lymphocytes; ●, CD4+ T lymphocytes; *, CD3+ T lymphocytes.

Fig. 4 Control of $SHIV_{SF162P4}$-replication during acute and chronic infection. ***a***, Plasma viral load levels; ***b***, Inverse correlation between the peak plasma viral load levels during acute infection (red bars) and the anti-$SHIV_{SF162P4}$ neutralizing potency of vaccine-elicited antibody responses at the day of viral-challenge (blue bars); ***c* and *d***, Development of anti-HIV envelope binding (***c***) and anti $SHIV_{SF162P4}$ neutralizing (***d***) antibody responses following infection; ***e*** CD4+ T cell numbers in the periphery following infection. Control animals: M844 ▲ and C640, ✳. Vaccinated animals I708 ▼, K863 ♦, N472 ◦ and P655 ■. (†) Indicates the time of death.

Fig. 5 Cellular anti-viral responses following immunization and during $SHIVS_{F162P4}$ infection. ***a***, Anti-SIVGag-directed responses; ***b***, anti-HIV envelope-directed responses; and ***c***, SEB-mediated responses were determined using the ELISPOT assay using cryopreserved PBMC collected two weeks following the end of the immunization protocol (Vaccination) and the indicated times following $SHIV_{SF162P4}$ infection from the control (M844 ■ and C640 ■) and vaccinated (I708 ▼, K863 ■, N472 ◦ and P655 ■) animals.

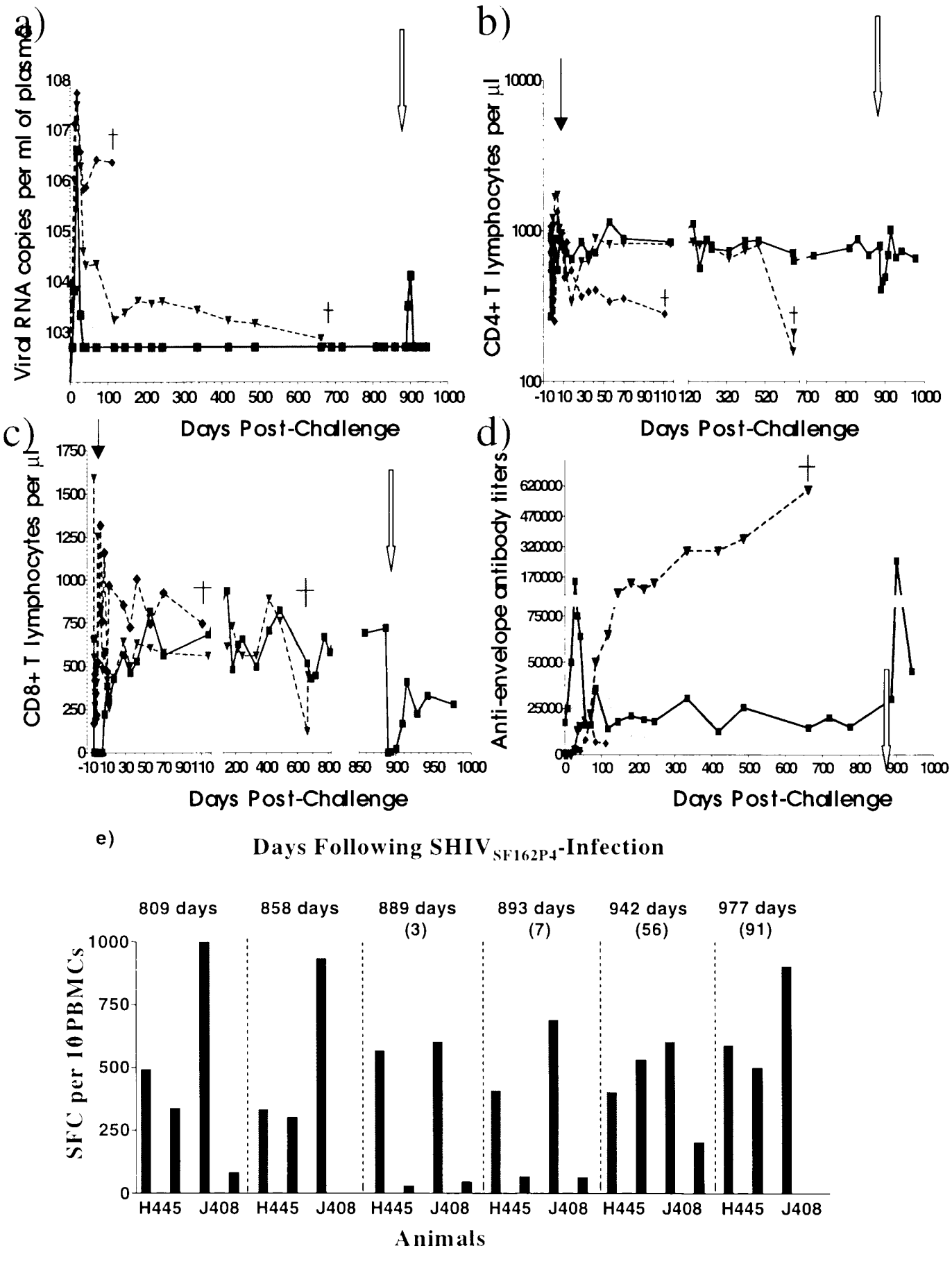

a)
Viral RNA copies per ml of plasma
108
107
106
105
104
103
0 100 200 300 400 500 600 700 800 900 1000
Days Post-Challenge
b)
CD4+ T lymphocytes per µl
10000
1000
100
-10 10 30 50 70 90 110 120 320 520 700 800 900 1000
Days Post-Challenge
c)
CD8+ T lymphocytes per µl
1750
1500
1250
1000
750
500
250
0
-10 10 30 50 70 90 110 200 400 600 800 850 900 950 1000
Days Post-Challenge
d)
Anti-envelope antibody titers
620000
470000
320000
170000
75000
50000
25000
0
0 100 200 300 400 500 600 700 800 900 1000
Days Post-Challenge
e)
Days Following SHIV SF162P4-Infection
809 days
858 days
889 days (3)
893 days (7)
942 days (56)
977 days (91)
SFC per 10 PBMCs
1000
750
500
250
0
H445 J408 H445 J408 H445 J408 H445 J408 H445 J408 H445 J408
Animals

Fig 1

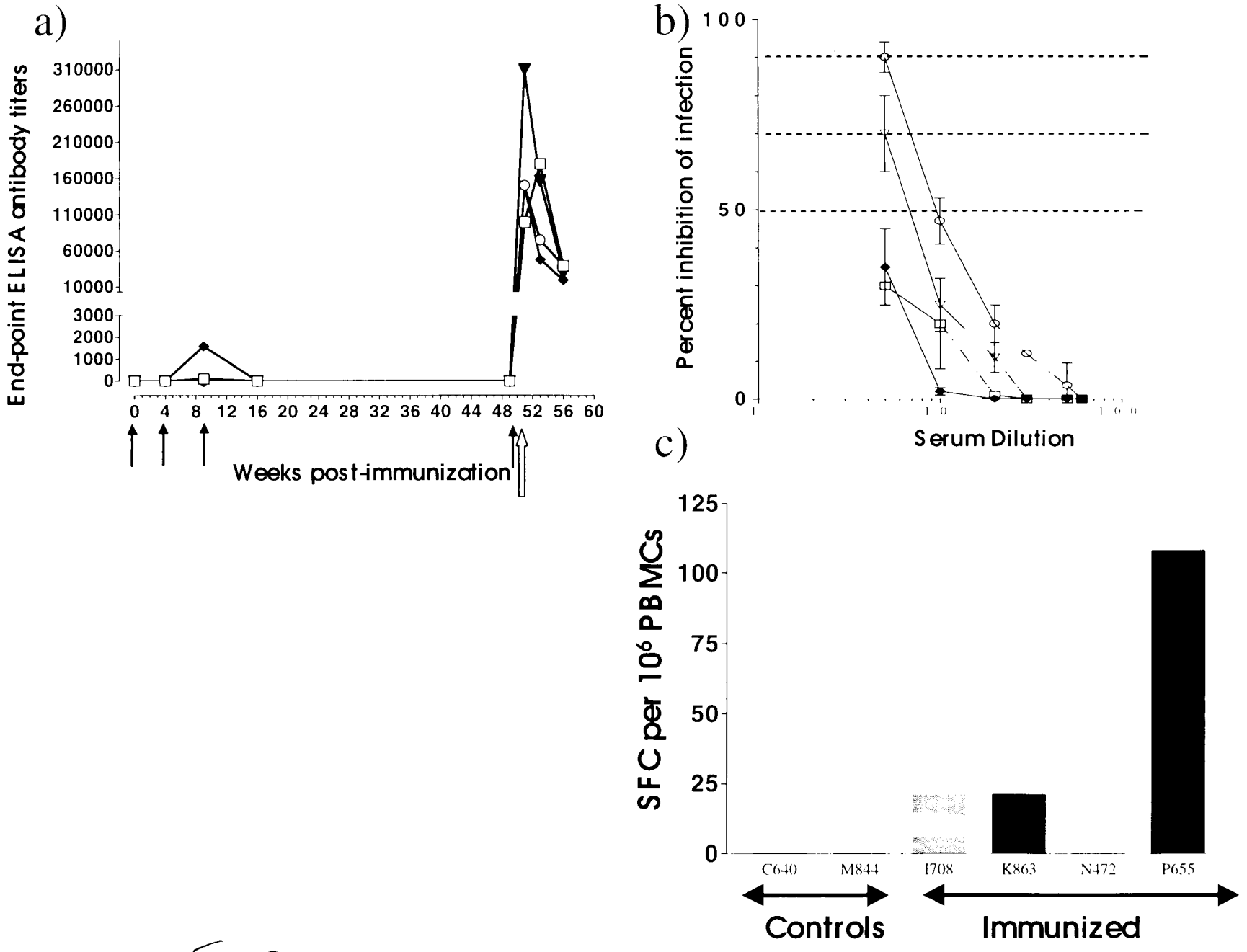

a)
End-point ELISA antibody titers
310000
260000
210000
160000
110000
60000
10000
3000
2000
1000
0
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60
Weeks post-immunization
b)
Percent inhibition of infection
100
50
0
Serum Dilution
c)
SFC per 10⁶ PBMCs
125
100
75
50
25
0
C640
M844
I708
K863
N472
P655
Controls
Immunized

Fig 2

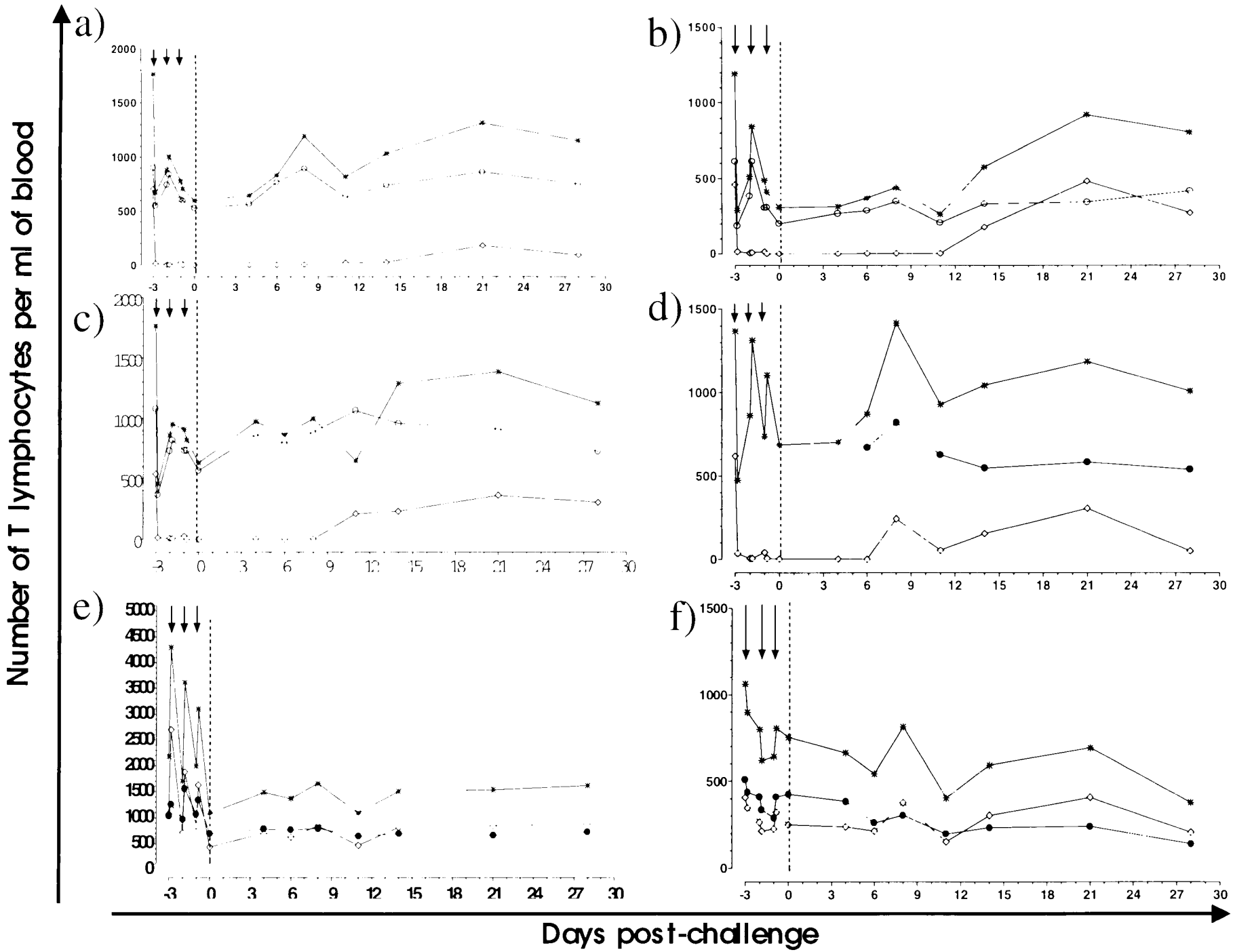

a)
b)
c)
d)
e)
f)
Number of T lymphocytes per ml of blood
Days post-challenge

Fig 3

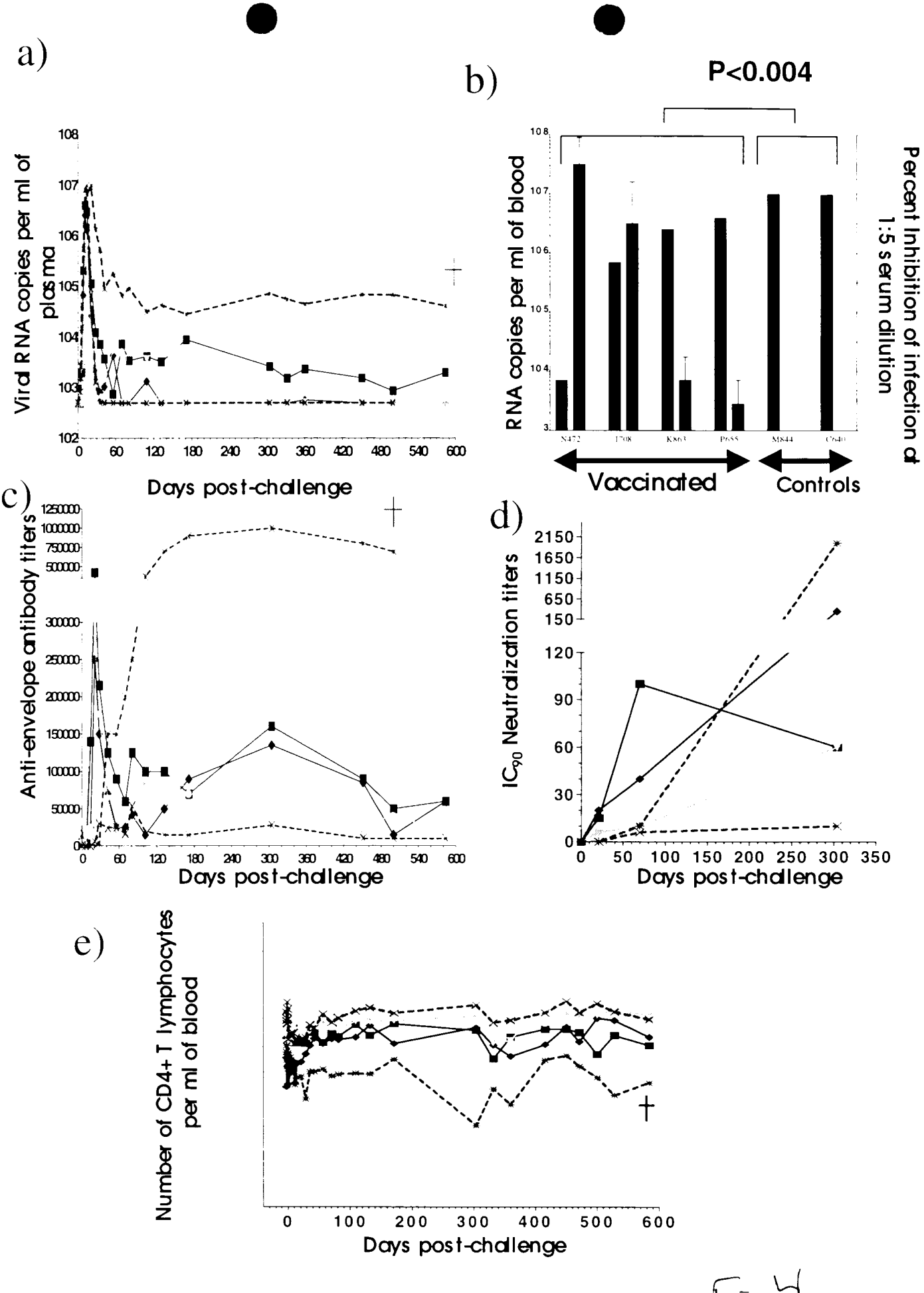
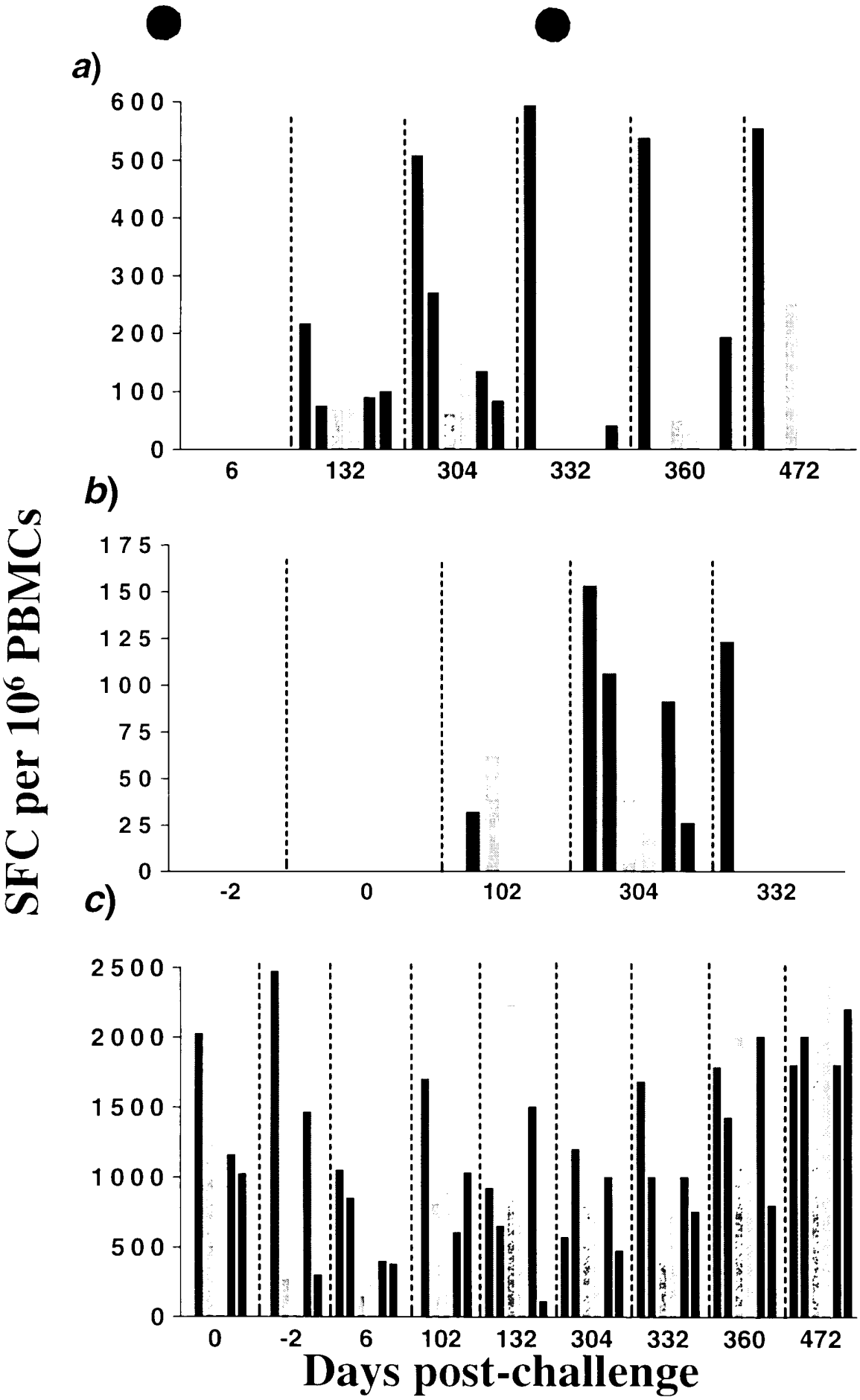

a)
Viral RNA copies per ml of plasma
Days post-challenge
b)
P<0.004
RNA copies per ml of blood
Percent Inhibition of infection at 1:5 serum dilution
N472
T308
K863
P655
M844
C640
Vaccinated
Controls
c)
Anti-envelope antibody titers
Days post-challenge
d)
IC90 Neutralization titers
Days post-challenge
e)
Number of CD4+ T lymphocytes per ml of blood
Days post-challenge

Fig 4


a)
600
500
400
300
200
100
0
6
132
304
332
360
472
b)
175
150
125
100
75
50
25
0
-2
0
102
304
332
c)
2500
2000
1500
1000
500
0
0
-2
6
102
132
304
332
360
472
SFC per 10⁶ PBMCs
Days post-challenge

F.g 5

EXHIBIT C

J Immunol Methods 1998 Nov 1;220(1-2):93-103 Related Articles, Links

Intranasal immunization with a plant virus expressing a peptide from HIV-1 gp41 stimulates better mucosal and systemic HIV-1-specific IgA and IgG than oral immunization.

Durrani Z, McInerney TL, McLain L, Jones T, Bellaby T, Brennan FR, Dimmock NJ.

Department of Biological Sciences, University of Warwick, Coventry, UK.

Control of pandemic human immunodeficiency virus type 1 (HIV-1) infection ideally requires specific mucosal immunity to protect the genital regions through which transmission more often occurs. Thus a vaccine that stimulates a disseminated mucosal and systemic protective immune response would be extremely useful. Here we have investigated the ability of a chimeric plant virus, cowpea mosaic virus (CPMV), expressing a 22 amino acid peptide (residues 731-752) of the transmembrane gp41 protein of HIV-1 IIIB (CPMV-HIV/1), to stimulate HIV-1-specific and CPMV-specific mucosal and serum antibody following intranasal or oral immunization together with the widely used mucosal adjuvant, cholera toxin. CPMV-HIV/1 has been shown previously to stimulate HIV-1-specific serum antibody in mice by parenteral immunization. All mice immunized intranasally with two doses of 10 microg of CPMV-HIV/1 produced both HIV-1-specific IgA in faeces as well as higher levels of specific, predominantly IgG2a, serum antibody. Thus there was a predominantly T helper 1 cell response. All mice also responded strongly to CPMV epitopes. Oral immunization of the chimeric cowpea mosaic virus was less effective, even at doses of 500 microg or greater, and stimulated HIV-1-specific serum antibody in only a minority of mice, and no faecal HIV-1 specific IgA.

PMID: 9839930 [PubMed - indexed for MEDLINE]

EXHIBIT D

Vaccine 1997 Jun;15(8):884-7 Related Articles, Links

FULL-TEXT ARTICLE

Anti-HIV env immunities elicited by nucleic acid vaccines.

Shiver JW, Davies ME, Yasutomi Y, Perry HC, Freed DC, Letvin NL, Liu MA.

Department of Virus and Cell Biology, Merck Research Laboratories, West Point, PA 19486, USA.

Plasmid DNA vaccines encoding HIV-1 env were used to immunize mice and nonhuman primates. Plasmids were prepared that produced either secreted gp120 or full-length gp160. Mice immunized with gp120 DNA developed strong antigen-specific antibody responses, CD8+ cytotoxic T lymphocytes (CTL) (following in vitro restimulation with gp120-derived peptide), and showed in vitro proliferation and Th1-like cytokine secretion [gamma-interferon, interleukin (IL)-2 with little or no IL-4] by lymphocytes obtained from all lymphatic compartments tested (spleen, blood, and inguinal, iliac, and mesenteric lymph nodes). This indicated that systemic anti-gp120 cell-mediated immunity was induced by this DNA vaccine. Although similar antibody responses were observed in mice immunized by either intramuscular or intradermal routes, T cell responses were significantly stronger in mice injected intramuscularly. Rhesus monkeys immunized with both gp120 and gp160 DNAs exhibited significant CD8+ CTL responses, following in vitro restimulation of peripheral blood lymphocytes with antigen. These experiments demonstrate that DNA immunization elicits potent immune responses against HIV env in both a rodent and a nonhuman primate species.

PMID: 9234539 [PubMed - indexed for MEDLINE]